Dietary Use of Artemisia Herba Alba Asso as A Potential Coccidiostat Against Cæcal Coccidiosis: Haematological Parameters Variations.

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Dietary use of *Artemisia herba alba* Asso as a potential coccidiostat against cæcal coccidiosis: haematological parameters variations.

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Abstract:

This study consists of the evaluation of the anticoccidial effect of *Artemisia herba-alba* Asso during experimental coccidial infection. Four groups of 30 broiler chickens were formed: the negative control (G1), the positive control (G2), the infected Monensin-treated group (G3), and the infected Artemisia-treated group (G4). Each infected bird received orally 10^5 sporulated oocysts of *Eimeria tenella*. No mortality was recorded in both G1 and G4. Haematocrit levels showed great variations from the 7th day Post-Infection, especially in G2 (20.87%±5.77). By day 10 P-I, haematocrit recovery was rapid particularly in G4 (28.07%±1.50). Haemoglobin concentration also decreased significantly ($p<0.05$) in all infected groups by the 7th day P-I. The reduction was very marked in G2 (6.47g/dL±1.67) against (10.53 g/dL ±0.25) in G1, but less marked in G4 (8.05g/dL ±1.56). Results show the protective effect of *A. herba-alba* Asso by improving the lesion score and the haematological parameters affected during coccidian infection.

Keywords: Artemisia, Coccidiostatic effect, Lesion score, Haematology, Broilers.

Declarations

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Conflicts of interest/Competing interests:

The authors declare that they have no conflict of interest.

Availability of data and material: Not applicable.

Code availability: Not applicable.

Authors' contributions

All authors contributed to the study conception and design. Writing the original draft and revising methodology were performed by Messaï Ahmed. Reviewing the paper and analyzing data were performed by Redouane-Salah Sara. All authors read and approved the final manuscript.

Ethics approval

This study followed the international guidelines of animal care and use in research and teaching (NRC, 2011). All procedures performed in this research were approved by the ethics committee on the use of animals from the Institute of Veterinary Sciences-University of Constantine1.

Consent to participate: Not applicable.

Consent for publication: Not applicable.

Introduction

Because of their therapeutic properties, several species belonging to the genus *Artemisia* are increasingly attracting the attention of researchers. The genus *Artemisia* L. belonging to the Asteraceae family includes more than 200 species (Singh et al., 2012). Isolation and identification of the chemical structure in the early 1970s, for the first time, of artemisinin (Figure 1) from *Artemisia annua* (Covello et al., 2007) have made these Asteraceae a valuable source of active ingredients with various biopharmaceutical properties (Tilaoui et al., 2011), especially antiprotozoal activity (Machín et al., 2020). In poultry farms, coccidiosis of broiler chicken is one of the main diseases to be controlled (Acharya and Acharya, 2017). Our knowledge of this protozoosis is quite considerable, but it still results in large losses worldwide, estimated by (Williams, 1999) at more than two billion dollars.

The sensitivity of different chicken strains to coccidia is different, but the development of poultry farming was possible only through the incorporation in feeding-stuff anticoccidial additives (ionophores or synthetic products). Since the 1950s, anticoccidial drugs have been used to control coccidiosis. These substances are currently subject to strict legislation which should lead to their prohibition in the coming years (Taljanski-

Zygmunt et al., 1998). Furthermore, the appearance of resistant strains of coccidia, making most of the available substances ineffective.

Among the new strategies, the use of phytogenic feed additives is proposed (Habibi et al., 2016). They are products of plant origin used in animal feeding as non-nutrient substances to enhance their performance and health (Abbas, 2012). Recent studies have demonstrated the efficacy of natural products, which appear to affect the development of coccidia and may be promising as food additives (Yang et al., 2021). (Allen et al., 1997; Arab et al., 2006; Jiao et al., 2018) demonstrated the anticoccidial effect of two Artemisia species extracts, in experimentally infected chickens with different *Eimeria* species.

In the present study *A. herba-alba* Asso, a species widespread in Algeria is studied for its anticoccidial effect in chickens, experimentally infected with *Eimeria tenella*. The effects of the plant were compared with those of a coccidiostat (monensin sodium) widely used in poultry farming. The study focused on the main parasitological (Lesion score) and haematological parameters (haematocrit level, haemoglobin concentration), modified during cæcal coccidiosis infections. The study would also describe a simple method of using the plant for its exploitation by breeders who often use chemical anticoccidials without a veterinary prescription. To our knowledge, this is the first study conducted to examine comparatively the prophylactic effects of *A. herba alba* Asso leaves on experimentally challenged broilers in Algeria.

a **b**

Fig 1. Artemisinin (**a**) and Monensin sodium (**b**) structure

Material and methods

The experimental protocol followed in this study is consistent with the international guidelines of animal care and use in research and teaching (NRC, 2011). Our study was carried out within the Institute of Veterinary Sciences of Constantine. Algeria.

1. Plant material

The aerial parts of *Artemisia herba alba* Asso were harvested from the T'Kout region (Batna, Algeria). This region is located on a plateau at an altitude of 1.200m. The climate is almost desert with a cold winter and a hot summer (Figure 2). After drying in the shade, the aerial parts were finely cut before being mixed with the animal's feed.

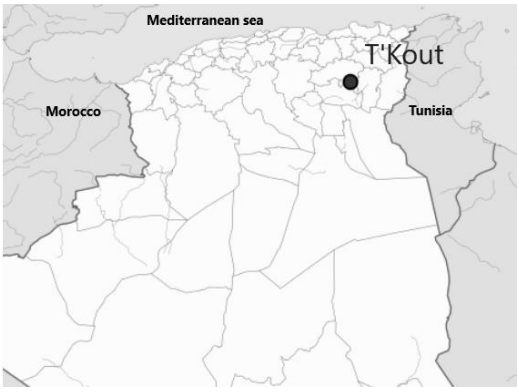

Fig 2. The location where *A. herba alba* Asso samples were collected

2. Parasite and inoculum preparation

A first inoculum, isolated from the litter of a broiler farm and kept in a 2.5% potassium dichromate solution in PADESCA laboratory, was multiplied to have a sufficient quantity of sporulated oocysts. Each milliliter of the solution contains an average of $24x10^3$ sporulated oocysts. On the 8th day after inoculation, the animals were autopsied and the intestinal masses recovered. The analysis proceeds then as follows: caeca were ground in distilled water and filtered through cheesecloth. The filtrate was centrifuged (3200t/min for 15min), and the pellets were re-suspended in saturated salt (NaCl) solution. After second centrifugation (3 minutes: the time required for oocyst flotation), the supernatants were re-suspended in distilled water to be washed, and then centrifuged again. Finally, all the pellets were recovered and kept in a 2.5% potassium dichromate solution. In a water bath, oocysts collected were kept at 30°C to undergo sporulation before they were infective. After 72 hours, sporulated oocysts were kept at +4°C until the day of inoculation. On the 18^{th} day of age (Day 0 of infection), animals of the infected groups G2, G3, and G4 received individually by gavage, 1 ml of a solution containing 10^5 sporulated oocysts of *Eimeria tenella* (sporulation rate 93.15%).

3 Animals

280 Hubbard-ISA[15] chicks of one day of age were bred under controlled hygiene conditions to prevent any contamination. Animals were vaccinated against Gumboro **IBA-VAC®**, and Newcastle diseases **BIO-VAC LA**

SOTA®. On the 17th day, four groups of 30 broiler chicken were formed (294.65±20.58g/subject): Negative control uninfected untreated group (**G1**), Positive control infected untreated group (**G2**), an infected group treated with Monensin (**G3**), and an infected group treated with *A. herba-alba* Asso (**G4**).

All feeds have been formulated to meet animal nutrient requirements (NRC, 1994). Standard ration (Corn 62%, Soybean cake 35%, Mineral and vitamin supplement 1%, Calcium carbonate 0.8%, bicalcic phosphate 1%, and sodium bicarbonate 0.02%) has been provided. Feed distributed to G1 and G2 was free of any anticoccidial supplementation, while feed distributed to groups G3 and G4 was supplemented with 100ppm of monensin sodium (Elancoban®) until the 45th day of age, and 5% dried leaves of *A. herba alba* Asso until the 30th day of age respectively.

The mortality rate was determined up to the 9th day post-infection. The lesion score was evaluated for 6 subjects per infected group at the 7th, 10 th, and 12 th days post-infection according to the method described by (Johnson and Reid, 1970). The haematological parameters analyses were carried out using an automaton, with kits (SPINREACT®). Haemoglobin concentration and Haematocrit were measured for 06 subjects from each group of animals. Blood samples were delicately obtained from the ulnar (wing) vein.

4. Statistical analysis

The statistical analysis was carried out by the Kruskal-Wallis test followed by the Mann-Whitney test of the XLSTAT 2010 software (Addinsoft SARL). The difference was considered significant when $p<0.05$. Treatment groups were compared to the Negative control uninfected untreated group (**G1**) and the Positive control infected untreated group (**G2**) for statistical difference ($p<0.05$).

Results

1. Mortality

Mortality rates are given in table 1.

Table 1: Mortality rates until 9days Post-Infection (n=06).

Experimental groups	**G1**	**G2**	**G3**	**G4**
Subjects dead	0/30	3/30	1/30	0/30
%	0	10	3.33	0

132 ***2. Lesion score***

133 The results reported in Table 2 represent arithmetic means obtained from the numerical values (0, 1, 2, 3, or 4)

134 attributed to the cæcal lesions.

135 **Table 2:** Lesion score recorded in the infected groups.

Post Infection Period (days)	Infected		
	G2	**G3**	**G4**
7	2.83±0.98[a]	2.33±0.52[a]	2.33±0.82[a]
10	1±0[a]	0.33±0.58[a]	1±0[a]
12	0.67±0.58[a]	0±0[a]	0.33±0.58[a]

136 Values are expressed as mean ± SEM (n=6). a, b: Values in the same row with a superscript in common do not

137 differ significantly ($p<0.05$).

138

139 ***3. Haematological parameters***

140 Table 3 present the results of the haematocrit and haemoglobin analysis. Blood samples were taken from 6

141 subjects from each experimental group.

142 **Table 3:** Haematocrit (%) and Haemoglobin concentration (g/dL) in experimental groups.

Post Infection Period (Days)	Uninfected	Infected		
	G1	**G2**	**G3**	**G4**
Haematocrit				
7	30.47±0.55[a]	20.87±5.77[b]	23.03±2.58[b]	23.70±3.84[b]
10	/	27.57±1.42[a]	27.47±2.89[a]	28.07±1.50[a]
12	28.63±4.45[a]	29.37±2.63[a]	32.03±1.12[a]	28.17±0.65[a]
Haemoglobin				
7	10.53±0.25[a]	6.47±1.67[b]	7.47±0.94[b]	8.05±1.56[b]
10	/	8.90±0.36[a]	8.90±1.15[a]	9.43±0.68[a]
12	9.63±1.40[a]	9.70±0.98[a]	10.47±0.29[a]	9.20±0.4[a]

143 Values are expressed as mean ± SEM (n=6). a, b: Values in the same row with a superscript in common do not

144 differ significantly ($p<0.05$).

145

146

Discussion

During the post-infection period, clinical signs of cæcal coccidiosis were observed in all infected chickens; immobility, weakness, bloody diarrhoea. The latter is pathognomonic during coccidial infections due to *E. tenella* (Conway and McKenzie, 2008). Among the infected groups, the less severe clinical signs were observed in Artemisia-treated animals (G4). Mortality rates were 10% and 3.33% respectively for the positive control (G2) and monensin-treated animals (G3), but no cases of mortality were recorded in the negative control (G1) and Artemisia-treated group. When added at 5% in the animal's feed, *A. herba alba* Asso dried leaves protected infected chickens from mortality and pathological symptoms caused by *E. Tenella*.

Lesion scoring is a technique developed to provide a numerical ranking of gross lesions caused by coccidian (Johnson and Reid, 1970). The cæcal lesions revealed at the autopsy were pathognomonic of a coccidial infection with *Eimeria tenella*: cæcums distended by bloody faeces and mucous debris, with haemorrhages on the mucosa. Such findings have been reported by several authors (Kadhim, 2014; Chen et al., 2020), in cases of cæcal coccidial infections. In our study, dried aerial parts of *A. herba-alba* Asso, incorporated in the diet reduced the cæcal lesions in infected chicks. Nevertheless, although numerically lower, the lesion score recorded in the Artemisia-treated group was not statistically different ($p<0.05$) from that observed in the positive control group. However, monensin has proven to be the most effective molecule in improving the lesion score, especially by the 12^{th} day post-infection. This carboxylic polyether ionophore, by its coccidicide effect, limits parasitic development (Moraes et al., 2019), resulting in faster regeneration of cæcal lesions.

Our results are in agreement with those reported in multiple studies, which have shown the beneficial effect of certain plants and their extracts on the improvement of the lesion score during *Eimeria tenella* coccidial infections (Papazahariadou et al., 2010; Yang et al., 2021).

Previous studies indicated that many biological impacts have always been reported during chicken coccidiosis including haematological changes (Akhtar et al., 2015), and various plasma metabolites. Due to haemorrhagic lesions following parasitic development, disturbance of erythrocytic parameters has been studied in our work, including haematocrit and haemoglobin concentration. In all the infected groups, the haematocrit level varied strongly from the 7^{th} day post-infection, especially in the positive control group (20.87±5.77). Decreased haematocrit may reflect anaemia in most cases. It can have different causes: iron deficiency, inflammation, intestinal malabsorption, or excessive blood loss (Janssens, 2009).

Furthermore, the occurrence in infected groups of diarrhoea with malabsorption, the inflammatory and haemorrhagic nature of the cæcal lesions may explain the significant fall ($p<0.05$) in haematocrit, especially on

177 the 7th day post-infection. With *Eimeria tenella*, parasitic development leads to ulcerative lesions in the cæcums,
178 and blood loss is almost constantly observed (Chandrakesan et al., 2009), hence the alteration of haematological
179 parameters and the appearance of signs of anaemia. On the 10th day P-I, recovery of haematocrit was rapid in
180 Artemisia-treated group but without significant difference from the positive control one.
181 As was observed for haematocrit, in all infected groups the haemoglobin concentration decreased significantly
182 from the 7th day post-infection. This reduction was very marked in the positive control Group (6.47±1.67)
183 compared with the negative control Group (10.53±0.25). These results are in agreement with those reported
184 recently by (Aljedaie and Al-Malki, 2020) who reported a significant decrease in haemoglobin concentration
185 (Hb) in chicks infected with *E. tenella* oocysts. In our study, haemorrhagic diarrhoea detected from the 5th day
186 post-infection, as well as the cæcal lesions revealed at autopsy, were accompanied by the appearance of anaemia
187 in infected chickens. In Artemisia-treated animals, the haematocrit and haemoglobin concentration were
188 numerically better than those recorded in animals of the positive control group. This finding could be due to the
189 protection conferred on animals by the bioactive constituents of the plant. Protection resulted in a reduction in
190 the haemorrhagic nature of the infection, confirmed by an improvement in the lesion score.
191 Based on current knowledge, it could be assumed that the attenuation of the severity of cæcal lesions, and the
192 improvement of haematological parameters during *E. tenella* by phytogenics is attributed mainly to artemisinin
193 (Dragan et al., 2010; Del Cacho et al., 2010), camphor and 1,8-cineole (Allen et al., 1997), tannins (Zaman et al.,
194 2012), and antioxidant compounds (Almeida et al. 2012; Lu et al., 2019; Zhang et al., 2020). The content of *A.*
195 *herba-alba* Asso in these compounds has been reported by several authors (Feuerstein et al., 1988; Messaï et al.,
196 2008; Bezza et al., 2010) suggesting that the plant we studied contains one or more of these compounds,
197 explaining the improvement of the lesion score in the Artemisia-treated group. However, among all these
198 bioactive compounds, artemisinin is the most studied. Many reports, suggested that the functional endoperoxide
199 bridge of artemisinin induces oxidative stress by generating a cascade of free radicals. The key point in the
200 antiprotozoal activities of artemisinin is the production of free radicals, and subsequently alkylation of proteins
201 and lipid peroxidation (Meshnick, 2002; Kaboutari et al., 2013; Pirali Kheirabadi et al., 2014). Furthermore,
202 artemisinin can slow down the reproduction of *E. tenella* and subsequently decrease the sporulation and survival
203 capability of the oocysts in the litter (Del Cacho et al. 2010). In addition to its direct action on *Eimeria*
204 development, artemisinin may block proinflammatory factors activated by the parasite (Pirali Kheirabadi et al.,
205 2014).

Through our study, the anticoccidial effect of *Artemisia herba alba* has not been completely understood. It seems that the plant may exert its effects via direct or indirect action of its active compounds on *Eimeria tenella* development.

Besides, in many studies on the anticoccidial effect of medicinal plants, authors have proven the effectiveness of certain plants in attenuation of the haemorrhage accompanying the development of *Eimeria tenella*, without identifying the active ingredient responsible for its effectiveness (Youn and Noh, 2001; Giannenas et al., 2003; Du and Hu, 2004). So, the beneficial effect of some of the unidentified compounds of the plant we have studied is also likely.

The prophylactic effects of *A herba alba Asso* have been examined in our study on *Eimeria tenella*-challenged broilers. Based on our results, it may be concluded that the addition of *Artemisia herba-alba* Asso at 5% to the broiler's diet has a positive effect on lowering the bloody diarrhoea intensity. In the search for alternatives to conventional anticoccidial drugs, our findings are encouraging. The effect of *A. herba alba* Asso was comparable to that of traditional prophylactic anticoccidial drugs: reduction of mortality rate, prevention of collapse of haematological parameters. This plant deserves to be the subject of more in-depth studies for better exploitation of its properties. As a first step in applying our results, the plant may be indicated to be exploited by farmers to reduce the economic impact of coccidiosis.

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